

# NOAA Technical Memorandum NMFS



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## **AN EVALUATION OF OOCYTE SIZE IN MULTIPLE REGRESSIONS PREDICTING GONAD WEIGHT FROM BODY WEIGHT: A TEST USING HAWAIIAN EHU, *Etelis carbunculus***

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U.S. DEPARTMENT OF COMMERCE  
National Oceanic and Atmospheric Administration  
National Marine Fisheries Service  
Southwest Fisheries Science Center

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**AN EVALUATION OF OOCYTE SIZE IN  
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GONAD WEIGHT FROM BODY WEIGHT:  
A TEST USING HAWAIIAN EHU, *Etelis carbunculus***

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## ABSTRACT

The interrelations of body size, ovary weight, and size of oocytes (ovarian eggs) were evaluated for 40 specimens of the Hawaiian snapper, *Etelis carbunculus*. The potential contribution of oocyte size (volume) for predicting ovary weight from body weight was assessed in multiple regressions relating ovary and body weights, with or without several measures of egg volume. Egg volume was first characterized using the median diameter of 25 oocytes in the largest, most advanced size mode present in each fish specimen. Weighted mean egg volumes were next estimated for each fish based on the diameters of 300 oocytes of all sizes, measured in increments of 50. Finally, the numbers of oocytes measured were evaluated in terms of benefit (gain in precision) versus cost (processing time).

In the basic model, ovary-free body weight (OFBW) explained 58% of the variation in gonad weight (GW) after each variable was transformed to logarithms. Addition of oocyte volume as a second independent variable contributed another  $38 \pm 1\%$  to the explained variance in GW. Egg volume based on the simple median diameter of the 25 largest oocytes was no less precise than egg volume characterized using the more time-consuming measurements of 50-300 diameters of all-sized oocytes. It is likely that larger (especially hydrated) oocytes have a disproportionate influence on ovary weight. The implications of reproductive cycling and allometric ovary-body weight relations are discussed. We conclude that the potential benefit of using the simply estimated egg volume measure may be greater for other ehu samples,

populations, or species in which the incidence of hydration is greater.

## INTRODUCTION

Five basic approaches have been used to determine the stage of gonad development of individual fish; these differ in precision, cost, and time of processing. (1) Histological staging of the most advanced size mode of oocytes is the most accurate but is also the most time consuming and expensive (Delahunty and DeVlaming, 1980). (2) Using light microscopy to measure sizes of the largest size class of oocytes effectively identifies the most advanced mode but may be inaccurate for ovaries that also contain oocytes in transitional stages of development. (3) Microscopic classification of whole oocytes can be applied with total confidence only when the developmental dynamics of oocytes is known for the particular species. (4) Macroscopic staging is a quick and gross method that is subjective and often unable to distinguish between immature and spent (resting) mature ovaries (West, 1990). (5) Determining the interrelations of ovary weight and body size, often but not always expressed as an index (Garcia-Berthou and Moreno-Amich, 1993) called the gonadosomatic index (GSI), is the least laborious (West, 1990). However, essential assumptions are either ignored or not explicitly addressed in most studies (DeVlaming et al., 1982).

Usually only a single method is used in a given study to assess the size at maturity of teleost fishes. Relatively few studies (e.g., Mackay, 1973; Jean and Lee, 1984; Cayré and Labrè, 1986; Hales, 1986; McQuinn, 1989; Hyndes et al., 1992; Hunter et

al., 1992) have attempted to confirm maturity classifications based on more than one technique. Some studies have identified size at maturity by combining light microscopic measurements of oocyte diameters with GSI results to produce a single more refined estimate (Schaefer and Orange, 1956; Higham and Nicholson, 1964; Yoshida, 1964; DeMartini and Fountain, 1981). None of the latter workers explicitly justified their mixture of methods. West (1990), however, recently identified microscopic staging and GSI as the most objective and quantitative method combination.

Several assumptions are basic to light microscopic measurements of oocyte diameters, the most important of which is that the numbers of oocytes measured provides acceptably precise estimates. Early studies used 200-300 oocytes per individual fish without justification. More recent studies have used fewer--usually 10-50 oocytes--for size measurements and other microscopic staging (West, 1990). In one apparently explicit justification, Yoshida (1964) reduced an initial sample size of 300 to 25 oocytes based on some ill-defined precision criterion.

The objectives of this study are twofold. First, we evaluate whether information on oocyte size improves ability to predict ovary weight from body weight. Given that oocyte size is important, we secondly evaluate the cost-effectiveness of different sample sizes for characterizing egg size. These considerations logically precede our use of data on ovary weight, adjusted for egg size, to derive gonad indexes that can be used

to identify reproductive readiness (sexual maturity) in ehu and other Hawaiian bottomfishes.

## MATERIALS and METHODS

### Field Sampling

Ehu (*Etelis carbunculus*, F. Lutjanidae) specimens were collected by hydraulic handline aboard the NOAA ship *Townsend Cromwell* at 70-120 fm depths near Necker Island, Nihoa Island, Kaula Rock, and French Frigate Shoals in the Northwestern Hawaiian Islands (NWHI). Collection occurred during June-September 1992-93, during the peak spawning season of ehu (Everson, 1984). These ehu were subsampled from a collection of ehu and other deepwater eteline snappers contributing to a larger study evaluating use of the GSI for characterizing size at maturity (DeMartini and Lau, unpubl. data).

Total wet body weight was measured to the nearest 0.01 kg. Fresh wet gonad weight was measured aboard ship ( $\pm 1$  g or  $\pm 10$  g for ovaries  $\leq 100$  or  $> 100$  g, respectively). A single sample of ovarian tissue (5-50 g, depending on ovary size) was taken from the central one-third of either the right or left ovary (random choice; Otsu and Uchida, 1959; Higham and Nicholson, 1964) and preserved in 10% formalin-sea water.

### Oocyte Measurements

Most specimens were processed within 1 to 2 months after collection. Four subsamples of approximately equal weight were randomly chosen from four different areas of the sample (Otsu and Uchida, 1959) and mixed in a petri dish. The diameters of 25 oocytes of the most advanced size mode were measured (random



axis; West, 1990) from the pooled subsamples. Then, the diameters of another 300 oocytes, randomly chosen from the pooled subsamples, were measured in lots of 50 oocytes. Diameters were recorded to the nearest ocular micrometer unit ( $= 0.0167$  mm, at  $63 \text{ omu} = 1 \text{ mm}$ ) at  $63\times$  magnification. The time to process the different-sized lots of oocytes was recorded.

### Data Analysis

Volumes of advanced and all-sized oocytes were calculated in two different ways. The median of the 25 diameters (most advanced size mode) was used to calculate a single, quick estimate of oocyte volume (volume,  $V = 4/3 \cdot \pi \cdot r^3$  in  $\text{omu}^3$ , where radius,  $r = \text{diameter}/2$ ). Mean volumes for the larger lots of all-sized oocytes were estimated in the following manner. First, a volume was calculated for each size class (1 omu class interval). Then, a weighted mean was derived by weighting the volume estimated for each size class by the frequency count in that class. The latter was done for each of the 300 oocyte samples in lots of 50 oocyte increments.

The statistical properties of both the original and double log-transformed (base 10) data for gonad weight (GW) and ovary-free body weight (OFBW; total weight less ovary weight) were first evaluated by bivariate scatterplots and least-squares mean regression. Plots were evaluated for nature of scatter and distribution of residuals. Following identification of the log-transformation as the more suitable data form (see Results), logarithms of the two potential independent variables (egg volume

and OFBW) were next regressed on one another in a test for collinearity. The explanatory power of the egg volume variable was then evaluated using a multiple regression model that included the egg volume variable:

$$\log_{10}Y = \log_{10}\alpha + \beta_1\log_{10}X_1 + \beta_2\log_{10}X_2 + \epsilon_{ij},$$

where  $Y = \text{GW (g)}$ ,  $\alpha = \text{the Y-intercept}$ ,  $\beta_1 = \text{the coefficient of } X_1$ ,  $X_1 = \text{OFBW (g)}$ ,  $\beta_2 = \text{the coefficient of } X_2$ ,  $X_2 = \text{egg volume (omu}^3\text{)}$ , and  $\epsilon_{ij} = \text{error}$ . Standardized residuals of the multiple regressions were used to identify possible outliers. Correlation coefficients were compared for models with and without the egg volume variable (Zar, 1984, p. 313). Correlation coefficients for the various log-based models including egg volume were tested for equality using the method of Zar (1984, p. 315):  $z$  transforms of the  $k$  regression coefficients were evaluated as chi-square with  $k - 1$  degrees of freedom. Regression analyses used PROC REG of the SAS PC release 6.03 (SAS Institute, 1988).

#### **Cost-Benefit Analysis**

For each differently measured and different-sized lot of oocytes measured, the benefit of including egg volume as a second independent variable was evaluated in terms of the proportion of variance in the dependent variable (gonad weight) that it explained. The cost of including egg volume was defined as the time required to process each different-sized lot of oocytes.

## RESULTS

### Fish, Ovary and Oocyte Relations

Forty ehu specimens with apparently developed ovaries underwent preliminary analyses. One specimen had a standardized residual of -3.887. As absolute values greater than 2 are considered statistical outliers (Cook and Weisberg, 1982, p. 18), this specimen was deleted from all further analyses using the 39 remaining fish.

If untransformed data were used, the relation between GW and OFBW was significant ( $R^2 = 0.30$ ,  $P < 0.001$ ; Table 1). However, the values of observations and the magnitude of their deviations from the regression line were obviously correlated (Fig. 1a), and residuals were badly skewed (Fig. 1b). Double log-transformation significantly improved the relation between GW and OFBW. The coefficient of determination ( $R^2$ ) increased by 27% over that for the regression using untransformed data (equation 1 vs equation 2; Table 1), with much reduced scatter among observations (Figs. 1a vs 1c). The double-log relationship was allometric, with a slope significantly different from 1 ( $H_0$ : slope = 1;  $F_{1,37} = 5.13$ ;  $P < 0.03$ ). The slope of a plot of the residuals was not significantly different than zero ( $H_0$ : slope = 0;  $R^2 = 0.0$ ;  $P = 1.0$ ).

Egg volume and OFBW were insignificantly related to one another, regardless of whether untransformed data ( $R^2 = 0.064$ ;  $P = 0.12$ ) or logarithms ( $R^2 = 0.053$ ;  $P = 0.16$ ) were used. This finding justified inclusion of log egg volume in the multiple

regression model. Adding egg volume (simply calculated using the median diameter of 25 of the largest size mode of oocytes) as a second independent variable explained an additional 39% of the variance in gonad weight (equation 2 vs equation 3; Table 1). The correlation coefficient significantly improved over the relationship based on OFBW alone ( $Z = 4.78$ ;  $P < 0.001$ ). Including the simple egg volume measure also reduced scatter in both the observations (Figs. 1c versus 2a) and the residuals (Figs. 1d versus 2b). The slope of the residuals of the log-based, multiple regression model (equation 3) was indistinguishable from zero ( $H_0$ : slope = 0;  $R^2 = 0.0$ ;  $P = 1.0$ ).

The analogous model, using egg volume calculated based on the weighted means for various numbers of all-sized oocytes, produced similar results (equations 4-9 vs equation 3; Table 1). Correlation coefficients were indistinguishable (0.97 - 0.98; Table 1) for both models (equations 3-9;  $\chi^2 = 1.946$ ;  $df = 6$ ;  $P > 0.9$ ).

### Processing Efficiency

The average times required to measure 300 oocytes in increments of 50 were linearly proportional to sample size. In contrast, the total time required to process 25 oocytes for a simple median diameter estimate was relatively greater, but absolutely less, than the time required to measure the smallest (50 oocyte) subsample for multiple estimates of weighted mean volumes (Table 2).

## DISCUSSION

### Reproductive Cycling and Allometry

Inclusion of egg volume as a second independent variable strengthened the quantitative relationship between gonad weight and body weight in ehu. Ovary weight in fishes is influenced by the stage of oocyte development (egg diameter and weight) because weights of oocytes and ovaries obviously increase during vitellogenesis. This may have particular significance for describing the reproductive state of serial spawners in which multiple batches of oocytes are sequentially ripened and shed in a spawning season. For such species, random collections of sample fish will invariably contain mixtures of females that have just spawned, are just about to spawn, or that are somewhere between these two extremes. Not surprisingly this is what we observed for ehu in the present study. Ehu are serial spawners, and the ovaries of reproductively active fish typically contain oocytes in varying stages of vitellogenesis (Everson, 1984).

Gonad weight is particularly influenced by body weight for fishes in which the relationship between the two variables is allometric (Reiss, 1987). Gonad-to-body weight relations in ehu are clearly allometric (slope =  $+1.47 \pm 0.21[\text{se}]$  for the logarithmic model; equation 2; Table 1). Such allometry, in which larger-sized females have disproportionately large ovaries, is typical of many teleosts (Erickson et al., 1984), and perhaps represents a physiological and evolutionary response to decreased reproductive value at older ages that correspond to larger sizes (Roff, 1983; Reiss, 1987). It is likely that both body size and

egg size have exaggerated effects on gonad size in many fishes besides ehu.

Body size and egg size together should explain much variation in ovary size for many fishes. Future studies relating ovary to body sizes in fishes should routinely evaluate some measure of egg size for improving the fit of gonad-to-body weight relations. Our findings in this study indicate that a simple measure of egg size can greatly improve fit.

#### **Benefits Versus Costs**

Using egg volume as a second independent variable, about 38% additional variance in gonad weight is explained beyond that provided by a model containing only ovary-free body weight. The benefit of a 38% increase in precision costs an additional 25 minutes per specimen, using the simplest measure of oocyte size. Measuring as few as 50 or as many as 300 oocytes of all sizes produced estimates of egg volume that were no more precise than that based on the simple median for 25 oocytes of the most advanced size mode. Clearly, the largest oocytes contribute disproportionately to ovary weight. Of the 39 ovaries examined, only five (13%) contained hydrated oocytes; however, the volume of hydrated oocytes on average was more than an order of magnitude greater than that of vitellogenic, but nonhydrated oocytes. It is likely that the influence of an egg size variable would be even greater for other sample populations, ehu stocks, or species in which the incidence of hydrated oocytes is greater.

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Table 1--Correlation coefficients and parameter estimates for Y (gonad weight, GW in grams), Y-intercept = (log)  $\alpha$ ,  $X_1$  = (log) OFBW (ovary-free body weight in grams),  $X_2$  = (log)  $EV_1$  (egg volume in  $omu^3$ ), and the errors ( $\epsilon_{ij}$ ) of the optional models tested. Logarithms used are base 10.

Models:		$R^2$	$\beta_1 \pm se$	$P > T$	$\beta_2 \pm se$	$P > T$	$a \pm se$	$P > T$
eqn1:	$GW = \alpha + \beta_1 OFBW + \epsilon_{ij}$	0.3048	$0.0567 \pm 0.01407$	0.0003	n/a	n/a	$-9.9694 \pm 17.3781$	0.5697
eqn2:	$\log GW = \log \alpha + \beta_1 (\log OFBW) + \epsilon_{ij}$	0.5765	$1.4690 \pm 0.20690$	0.0001	n/a	n/a	$-2.9126 \pm 0.6114$	0.0001
eqn3:	$\log GW = \log \alpha + \beta_1 (\log OFBW) + \beta_2 (\log EV_{25}) + \epsilon_{ij}$	0.9686	$1.1834 \pm 0.05875$	0.0001	$0.5764 \pm 0.02721$	0.0001	$-4.2651 \pm 0.1806$	0.0001
eqn4:	$\log GW = \log \alpha + \beta_1 (\log OFBW) + \beta_2 (\log EV_{50}) + \epsilon_{ij}$	0.9456	$1.2228 \pm 0.07683$	0.0001	$0.5728 \pm 0.03665$	0.0001	$-4.3162 \pm 0.2396$	0.0001
eqn5:	$\log GW = \log \alpha + \beta_1 (\log OFBW) + \beta_2 (\log EV_{100}) + \epsilon_{ij}$	0.9467	$1.2311 \pm 0.07594$	0.0001	$0.5829 \pm 0.03686$	0.0001	$-4.3859 \pm 0.2388$	0.0001
eqn6:	$\log GW = \log \alpha + \beta_1 (\log OFBW) + \beta_2 (\log EV_{150}) + \epsilon_{ij}$	0.9497	$1.2362 \pm 0.07373$	0.0001	$0.5819 \pm 0.03561$	0.0001	$-4.3967 \pm 0.2322$	0.0001
eqn7:	$\log GW = \log \alpha + \beta_1 (\log OFBW) + \beta_2 (\log EV_{200}) + \epsilon_{ij}$	0.9513	$1.2364 \pm 0.07250$	0.0001	$0.5797 \pm 0.03482$	0.0001	$-4.3915 \pm 0.2281$	0.0001
eqn8:	$\log GW = \log \alpha + \beta_1 (\log OFBW) + \beta_2 (\log EV_{250}) + \epsilon_{ij}$	0.9529	$1.2372 \pm 0.07129$	0.0001	$0.5787 \pm 0.03411$	0.0001	$-4.3924 \pm 0.2243$	0.0001
eqn9:	$\log GW = \log \alpha + \beta_1 (\log OFBW) + \beta_2 (\log EV_{300}) + \epsilon_{ij}$	0.9535	$1.2354 \pm 0.07094$	0.0001	$0.5768 \pm 0.03380$	0.0001	$-4.3811 \pm 0.2229$	0.0001

Table 2--Estimates of the mean times required to process oocyte samples of different sizes. Preparation time is that necessary prior to measurement of oocytes. Measuring time is that required to measure diameters. Total time is preparation time plus measuring time.

No. oocytes	Preparation time (min)	Measuring time (min)	Total time (min)
25	20	5	25
50	20	20	40
100	20	45	65
150	20	65	85
200	20	85	105
250	20	110	130
300	20	130	150

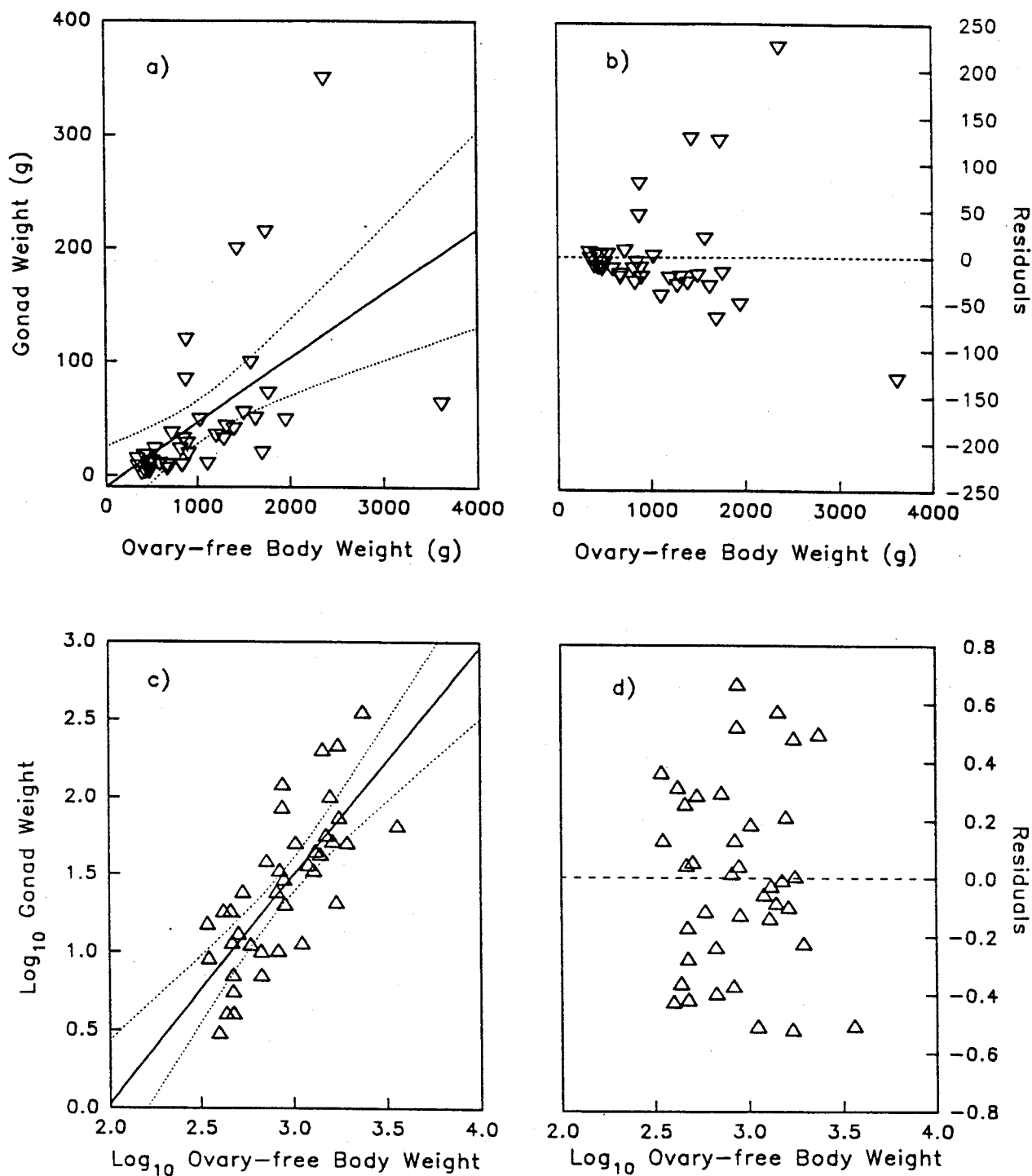


Figure 1--Scatterplots of (a) gonad weight regressed on ovary-free body weight (model:  $GW = \alpha + \beta_1 OFBW + \epsilon_{ij}$ ;  $R^2 = 0.305$ ;  $P < 0.001$ ); (b) residuals of (a) with zero line included; (c) a double-log plot of the same variables (model:  $\log_{10} GW = \log_{10} \alpha + \beta_1 \log_{10} OFBW + \epsilon_{ij}$ ;  $R^2 = 0.576$ ;  $P < 0.0001$ ); and (d) residuals of (c) with zero line included. Regression lines with 95% confidence interval bounds are illustrated.

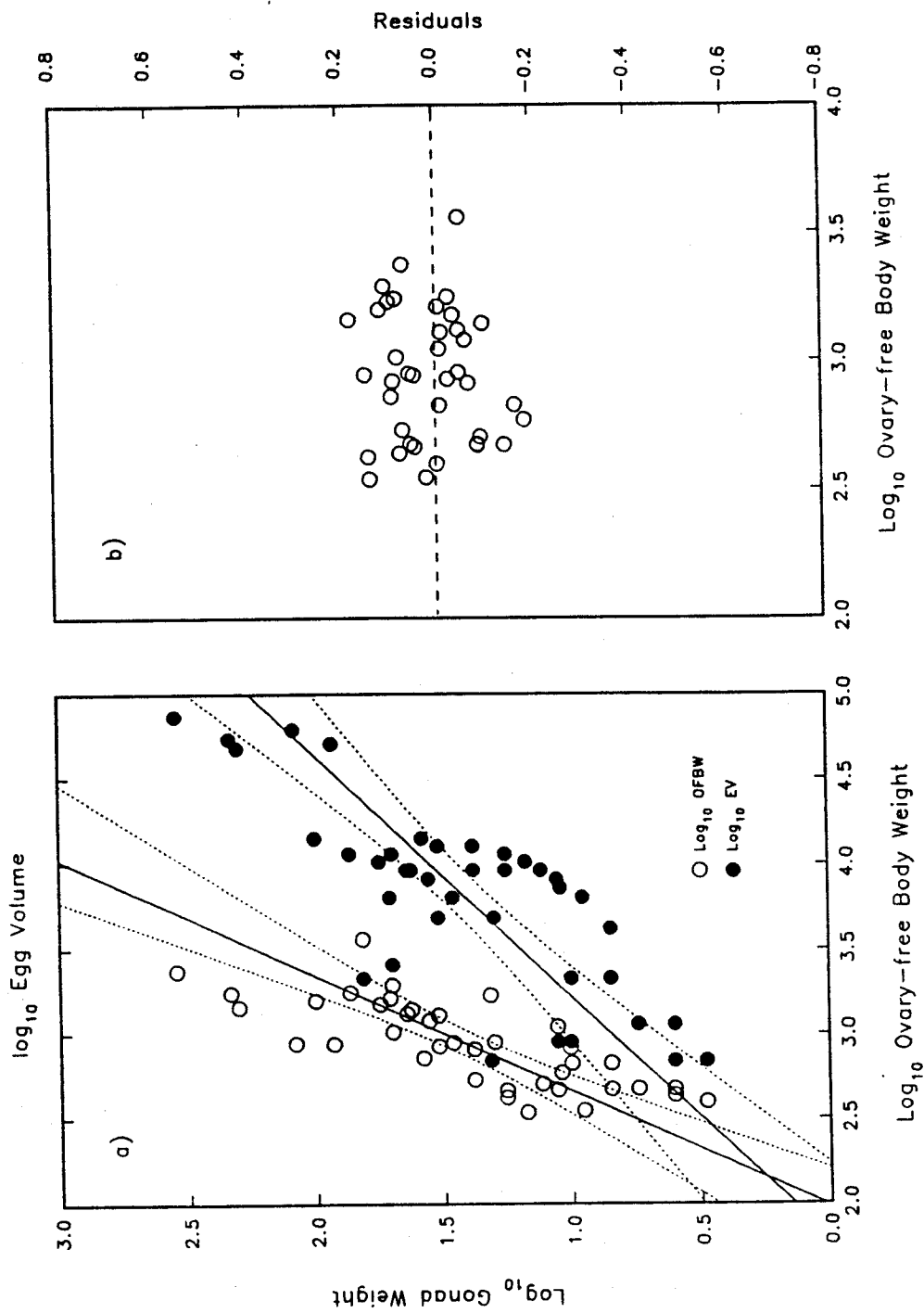


Figure 2--(a) Overlay plots of log gonad weight regressed on log ovary-free body weight (hollow circles) ( $R^2 = 0.305$ ;  $P < 0.001$ ) and log gonad weight regressed on log egg volume, with the latter based on the median diameter of 25 ova (filled circles) ( $R^2 = 0.614$ ;  $P < 0.0001$ ). Regression lines with 95% confidence interval bounds are shown. (b) Plot of residuals for the multiple regression model relating log gonad weight to log egg volume and log ovary-free body weight with zero line included (model:  $\log_{10}GW = \log_{10}\alpha + \beta_1\log_{10}OFBW + \beta_2\log_{10}EV_{25} + \epsilon_{ij}$ ).

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